THE CONTRIBUTIONS OF VARIOUS PATHWAYS TO DIKETOPIPERAZINE FORMATION FROM PEPTIDES CONTAINING ASPARTIC ACID RESIDUES IN THE PENULTIMATE POSITION

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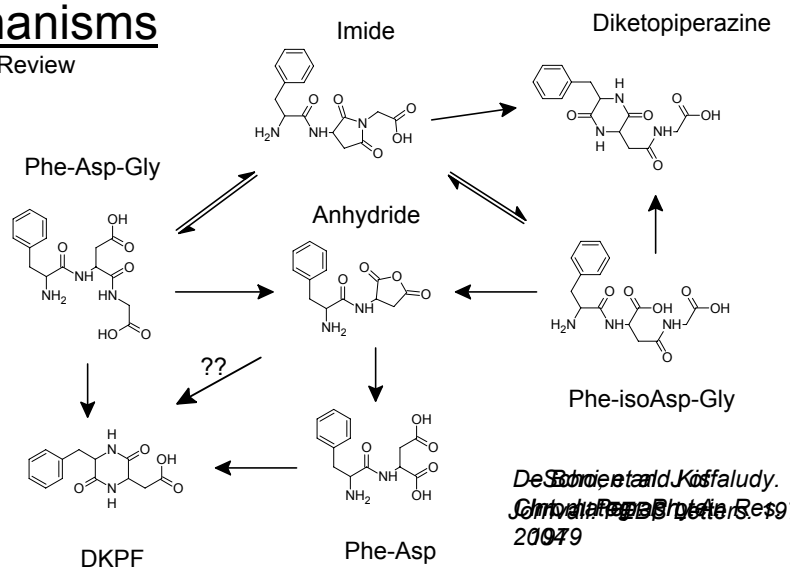
Protein Stability

- Aspartic Acid is a Hot spot
- Peptide Synthesis
- Peptide Formulation
  - Human Growth Hormone
  - Interleukin-11
- Disease States
  - Alzheimer's Disease
  - Cataract
  - Parkinson's Disease

Shimizu, Matsuoka, Shirasawa. *Biol. Pharm. Bull.* **28**(9) 1590-1596 (2005)

Mechanisms

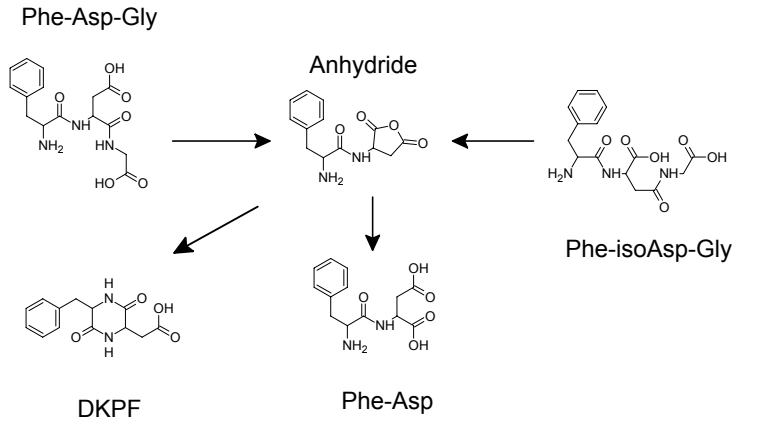
Literature Review



De Bont et al. Kiss et al. Int. J. Pept. Protein Res. 1974
Schroeder and Kossiakoff. Biochem. Biophys. Res. Commun. 1979

Mechanisms

New Mechanism



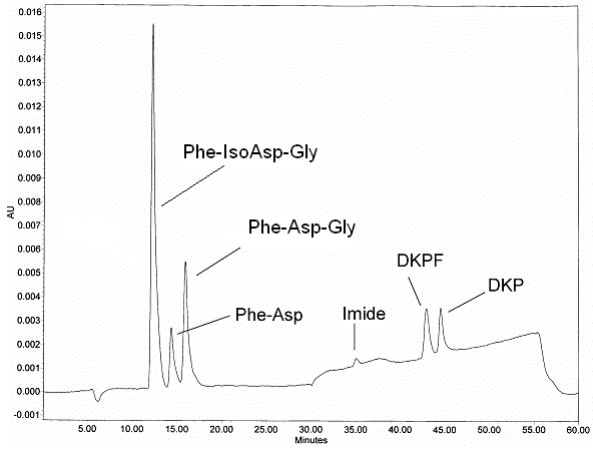
Hypothesis

- The anhydride generated during peptide fragmentation at aspartyl residues is susceptible to nucleophilic attack by the N-terminus.
- DKPF forms via the anhydride.
- pH will greatly affect the formation of DKPF and DKP

Materials and Methods

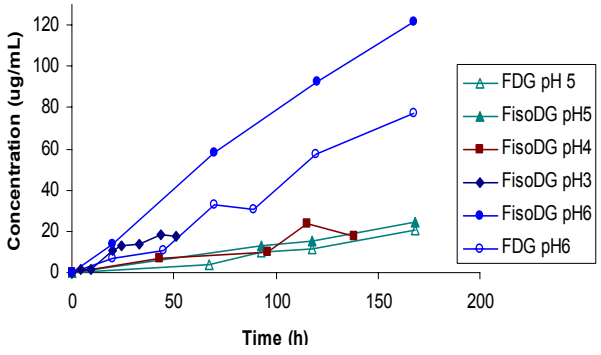
- 50mM Buffers from pH 3-6
- HPLC Analysis
  - isocratic; UV detection (257nm)
- Phe-Asp-Gly and Phe-isoAsp-Gly as Starting Compounds (200µg/mL)
- Differential Equations
  - Based on the Previous Scheme

HPLC Chromatogram



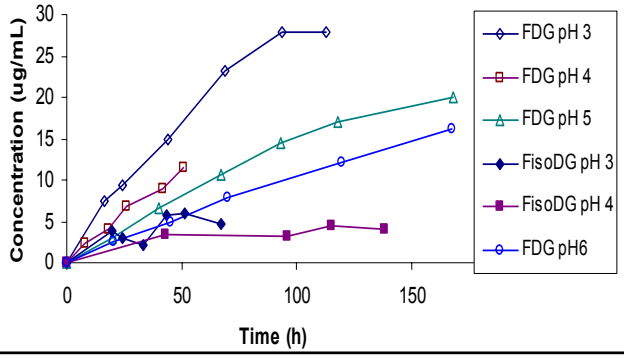
DKP Formation

- DKP formation is greatest when FisoDG is the starting compound
- U-shaped pH curve
- No DKP from FDG at acidic pH



DKPF Formation

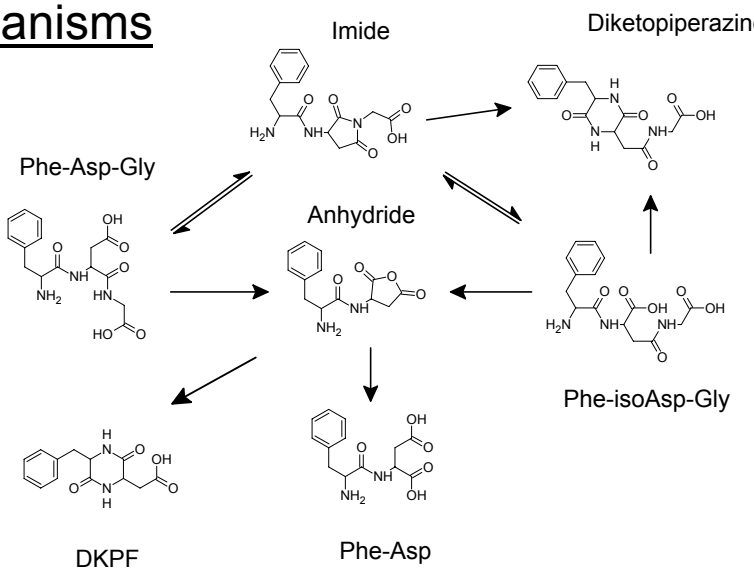
- DKPF formation is greatest when FDG is the starting compound
- Increase in pH decreases DKPF
- No DKPF from FisoDG above pH 4



Aspartate Degradation at pH 3.00

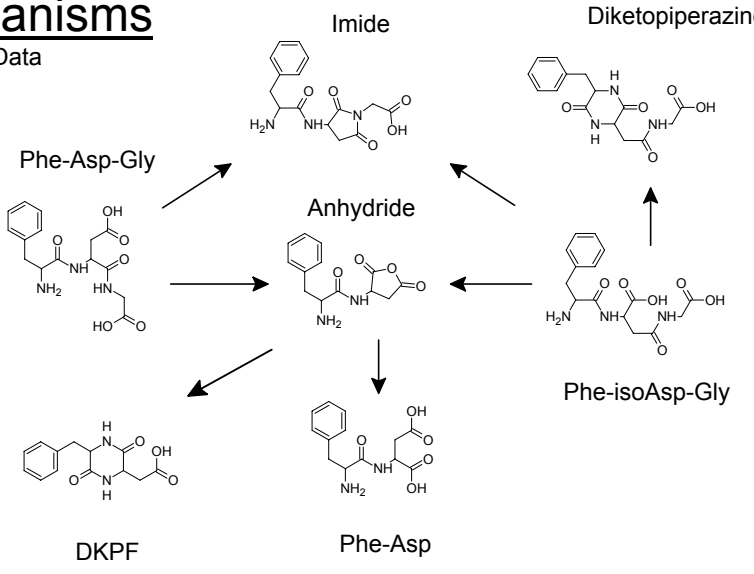
- Phe-isoAsp-Gly (FisoDG)
  - No isomerization to Aspartate
  - Formation of DKPF and DKP
  - Similar Rate of loss as FDG
- Phe-Asp-Gly (FDG)
  - Minimal isomerization to isoaspartate (<0.7%)
  - Formation of DKPF but no DKP
  - Similar Rate of loss as FisoDG

Mechanisms

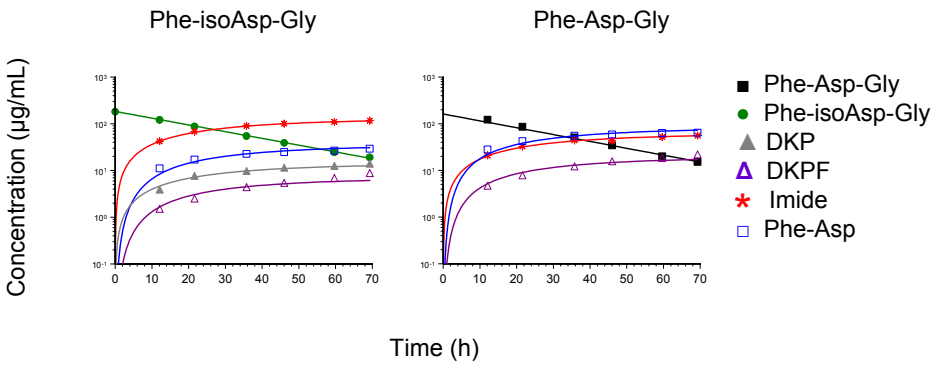


Mechanisms

Based on Data

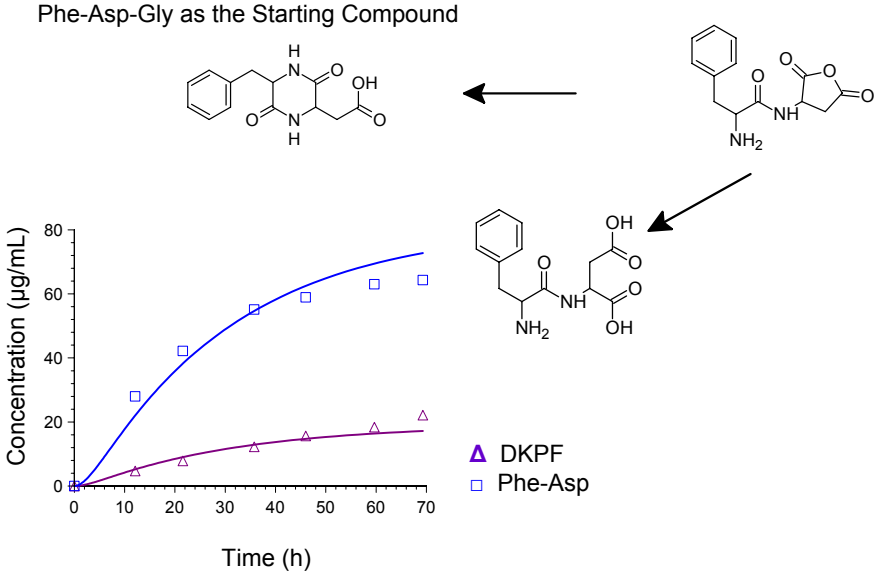


Aspartyl Peptide Degradation



Anhydride to DKPF and Phe-Asp

Phe-Asp-Gly as the Starting Compound



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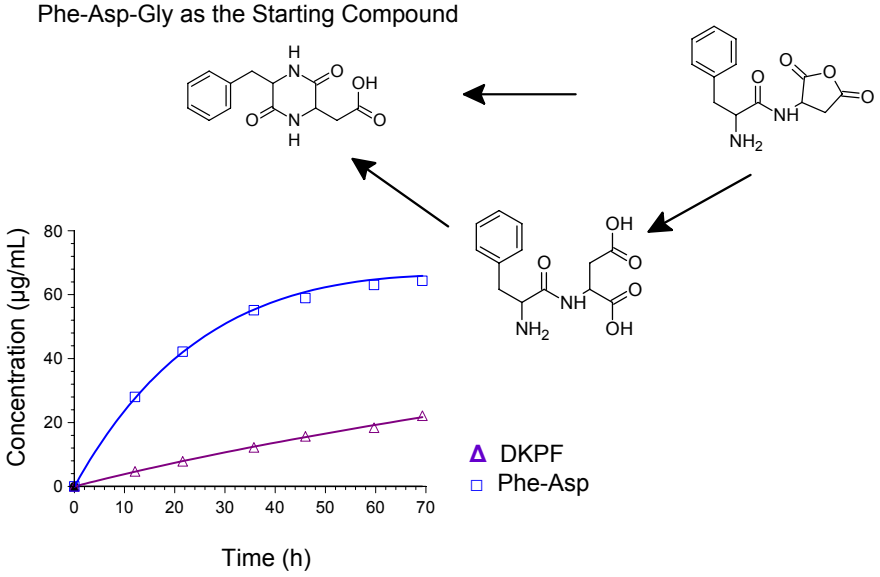
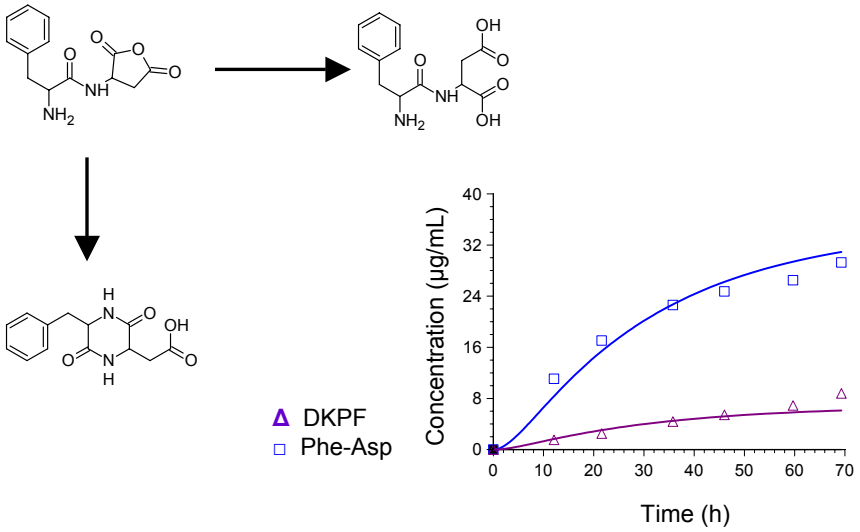


Anhydride to DKPF and Phe-Asp

Phe-isoAsp-Gly as the Starting Compound



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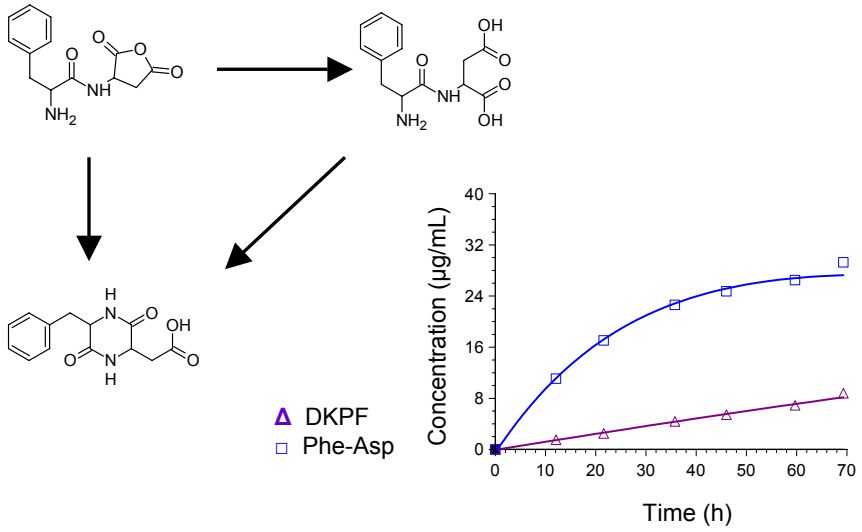
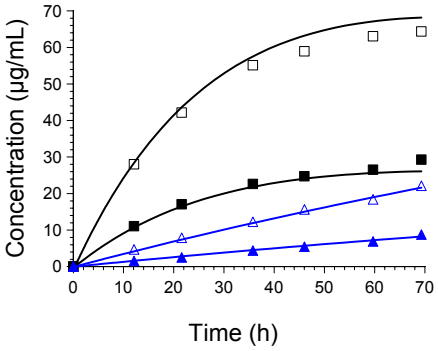


Addition of Parameters

- Fit Any Line
- Mechanism of Formation Based on Structures of the Compounds
- Simultaneous Fit
  - Combine the Phe-Asp-Gly and Phe-isoAsp-Gly Data
  - 9 Equations and 9 Parameters

Simultaneous Fitting



Plot of DKPF Formation from Anhydride and Phe-Asp

- □ - Phe-Asp from Phe-Asp-Gly
- ■ - Phe-Asp from Phe-isoAsp-Gly
- △ - DKPF from Phe-Asp-Gly
- ▲- DKPF from Phe-isoAsp-Gly

- Anhydride to DKPF Only
  - 0.618 SSD
- w/ Phe-Asp to DKPF
  - 0.183 SSD
- Above w/ Phe-Asp-Gly to DKPF
  - 0.137 SSD

Data Supporting Anhydride

- Anilide trapping of anhydride from dipeptide Asp-Phe
- Loss of Asp-Phe did not increase with increasing aniline concentration
- Found two anilide compounds
  - Alpha and Beta
- Formation of Phe did not decrease
  - Multiple pathways of degradation

Anjali Joshi, Ramil Menzeleev, and Biren Joshi. AAPS Poster, 2005

Conclusions

- Models Were Derived that Implicate the Anhydride Intermediate
- There are Multiple Pathways for DKPF and DKP Formation
  - DKPF – Anhydride and Phe-Asp cyclization
  - DKP – Imide and N-terminus/isoaspartate cyclization
- pH can turn off certain pathways
- Applications to proteins

Future Experiments

- Aniline Experiment
  - FDG and FisoDG as the starting compounds
  - Reduction of Phe-Asp and DKPF
- Phe-Asp to DKPF
  - Adds More Information to the Model
- Solid-State Kinetics
  - Intramolecular reaction dependent on Tg

Acknowledgements

- D. White
- Dr. Bradley D. Anderson – Advisor
- A Grant from Pfizer Inc.